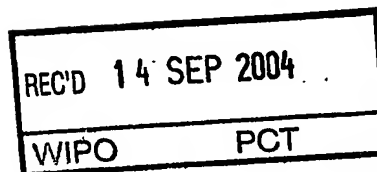


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CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 19 August 2003 with an application for Letters Patent number 527678 made by EDWIN LOWE; SKELTE ANEM and SIEW KIM LEE.

Dated 1 September 2004.

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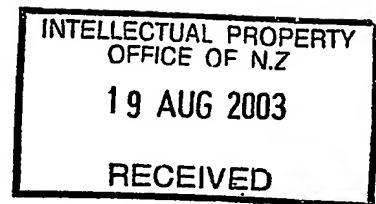
527678

NEW ZEALAND

PATENTS ACT, 1953

PROVISIONAL SPECIFICATION

DAIRY PRODUCT AND PROCESS



We, Edwin Lowe, a New Zealand citizen, Skelte Anema, a New Zealand citizen, and Siew Kim Lee, a Singaporean citizen of Fonterra Research Centre, Dairy Farm Road, Palmerston North, New Zealand, do hereby declare this invention to be described in the following statement:

FIELD OF THE INVENTION

The present invention involves the use of yoghurt texture improvers containing whey proteins in conjunction with pH adjustment and heat treatment, in yoghurt manufacture. Specifically the invention involves the control of consistency of yoghurt.

BACKGROUND

Yoghurt-making processes have developed over the years to improve the quality of the product delivered to consumers. In particular, it is important to ensure that yoghurt has a desirable texture and consistency.

To make yoghurt, milk is contacted with a lactic starter and then is fermented. Flavour develops over time and the intermediary "yoghurt milk" hardens to the desired gel-like texture, and is sold as yoghurt.

In modern yoghurt manufacture processes, yoghurt milk undergoes a heat treatment. It is during this heating step that beta-lactoglobulin is denatured, allowing it to bond to kappa-casein. Lucey & Singh (1998) suggested that the aggregation of denatured whey proteins with associated caseins at the isoelectric point of beta-lactoglobulin is the beginning of the gelation process.

On a commercial scale it is difficult to control the texture and consistency of the final product during the yoghurt-making process itself.

It is therefore an object of the invention to provide an improved or alternative method of preparing a yoghurt or yoghurt based drink.

SUMMARY OF THE INVENTION

In one aspect the invention provides a method of preparing a yoghurt comprising the following steps:

- adding an amount of whey protein to yoghurt milk at a pH below the natural pH of the yoghurt milk,
- heating the yoghurt milk,
- optionally adjusting the pH back to the natural pH of the yoghurt milk,
- acidifying the milk using a microorganism treatment or chemical acidification to prepare a yoghurt.

In a second aspect the invention provides a method of preparing a yoghurt comprising the following steps:

- adding an amount of whey protein to yoghurt milk
- adjusting the pH of the yoghurt milk to a pH below the natural pH of the yoghurt milk,
- heating the yoghurt milk,
- optionally adjusting the pH back to the natural pH of the yoghurt milk,
- acidifying the milk using a microorganism treatment or chemical acidification to prepare a yoghurt.

Preferably the yoghurt milk is adjusted to a preselected pH below the natural pH of the yoghurt milk.

Preferably more than 0.32 % w/w whey protein is added to the yoghurt milk.

More preferably 0.32% to 2% w/w whey protein is added to the milk.

Preferably the yoghurt milk is heated at between 70°C and the boiling point of the liquid milk.

Preferably the yoghurt milk is heated for 0.1 seconds to 60 minutes .

The more preferred duration of the heating step varies with the temperature used. Generally a time in the range of 10 seconds to 30 minutes is used.

Preferably the pH used for the heat treatment step is between the natural pH and 5.0.

More preferably the pH used for the heat treatment step is between 5.9 and 6.7.

Most preferably the pH used for the heat treatment step is between 6.3 and 6.7.

In a third aspect of the invention, there is described a method of preparing a yoghurt comprising .

- adding an amount of whey protein to yoghurt milk at a pH above the natural pH of the yoghurt milk,
- heating the yoghurt milk,
- optionally adjusting the pH back to the natural pH of the yoghurt milk
- acidifying the milk using a microorganism treatment or chemical acidification to prepare a yoghurt.

In a fourth aspect of the invention, there is described a method of preparing a yoghurt comprising

- adding an amount of whey protein to yoghurt milk
- adjusting the pH of the yoghurt milk to a pH above the natural pH of the yoghurt milk,
- heating the yoghurt milk,
- optionally adjusting the pH back to the natural pH of the yoghurt milk
- acidifying the milk using a microorganism treatment or chemical acidification to prepare a yoghurt.

Preferably the yoghurt milk is adjusted to a preselected pH above the natural pH of the yoghurt milk.

Preferably less than 0.32 % (w/w) whey protein is added to the yoghurt milk.

Preferably the yoghurt milk is heated at between 70°C and the boiling point of the liquid milk.

Preferably the yoghurt milk is heated for 0.1 seconds and 60 minutes .

The more preferred duration of the heating step varies with the temperature used. Generally a time in the range of 10 seconds to 30 minutes is used.

Preferably the pH used for the heat treatment step is between the natural pH and 8.0.

More preferably the pH used for the heat treatment step is between 6.9 and 7.5.

Most preferably the pH used for the heat treatment step is between 6.9 and 7.2.

The methods of the invention allow the preparation of yoghurts with altered gel strengths relative to corresponding methods without the pH adjustment.

This invention may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, and any or all combinations of any two or more of said parts, elements or features, and where specific integers are mentioned herein which have known equivalents in the art to which this invention relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

The invention consists in the foregoing and also envisages constructions of which the following gives examples.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the effect of pH at heat treatment on the final acid gel strength for reconstituted skim milk with no added whey protein.

Figure 2 is a graph comparing the effect of pH at heat treatment on the final acid gel strength where two different milk samples were used – whey protein (added as whey protein concentrate with 80% protein)-fortified reconstituted skim milk and non-fortified reconstituted skim milk.

Figure 3 is a graph showing the effect of heat treatment, pH and different levels of whey protein fortification.

Figure 4 is a graph showing the effect of whey protein addition / levels on the optimum heat-treatment pH.

DETAILED DESCRIPTION OF THE INVENTION

References to "yoghurt texture" include references to the texture of any intermediary gel produced during the yoghurt making process (e.g. acid gel strength).

Whey proteins are often added to yoghurt to improve its texture, water holding ability and mouthfeel. It is thought that the effect of whey proteins on yoghurt texture is related to the heat denaturation of the whey proteins, particularly β -lactoglobulin, in the presence of casein micelles.

The applicant has discovered that if whey protein- fortified milk is heated at a pH below or above its natural pH, then the texture of the resulting yoghurt is altered.

Where the whey protein fortification level is relatively high, the applicant has discovered that heating the yoghurt milk at a pH lower than the natural pH of the yoghurt milk results in a firmer yoghurt texture.

Where the whey protein fortification level is relatively low, the applicant has discovered that heating the yoghurt milk at a pH above the natural pH of the yoghurt milk results in a firmer yoghurt texture as the pH increases.

Furthermore, the applicant has discovered that for any given level of whey protein addition to yoghurt milk, there is an optimum pH for heat treatment that results in the firmest yoghurt texture.

Figure 1 shows that as the heat treatment pH of a non-fortified milk is increased, the texture of the final acid gel strength increases, but tapers off after the heat treatment pH reaches approximately 7.0.

Figure 2 shows that the whey protein fortified milk (in this example 10% reconstituted skim milk with 1.2% added 80% whey protein concentrate) results in increased acid gel strength with lower heat treatment pH. This is in stark contrast with the non-fortified milk example shown in both Figures 1 and 2.

Figure 3 shows that the whey protein concentration also affects the resulting acid gel strength. In this particular example, where the whey protein concentration was under 0.32 %, higher heat treatment pH levels resulted in higher acid gel strength. Where the whey protein concentrate was above 0.32 %, lower heat treatment pH levels resulted in higher acid gel strength.

Figure 4 demonstrates that for any given whey protein-fortified milk, the concentration of whey protein in the milk will affect what the optimal heat strength pH is (in order to give the highest acid gel strength).

As used herein, "WPC80" refers to a whey protein concentrate containing about 80% protein.

EXAMPLES

The following examples further illustrate the practice of the invention.

Example 1: Skim milk acid gels prepared from reconstituted skim milk. The gel strength of the acid gels can be varied depending on the pH at heating.

	Weight (g)
Low heat skim milk powder	100g
Water	900g

Milk preparation, pH adjustment and heating

Reconstituted skim milk samples were prepared by adding low heat skim milk powder (100g, whey protein nitrogen index above 6; 37% protein, Fonterra Co-operative Group, Pahiatua Manufacturing Site, New Zealand) to purified water (900g, purified by reverse osmosis followed by filtration through Milli-Q apparatus) to a final concentration of 10% (w/w) total solids. The reconstituted skim milk samples were allowed to equilibrate at ambient temperature (about 20 °C) for at least 10 h before further treatment.

The skim milk was separated into several sub-samples. The pH of the milk sub-samples was adjusted to a pH in the range 6.5 to 7.1 with either 3M HCl or 3M NaOH. The samples were allowed to equilibrate for at least 2 h and then minor re-adjustments were made. The pH-adjusted milk samples (50g) were placed in screw top glass bottles and heated at 80°C for 30 minutes in a water bath. After heat treatment, the milk samples were cooled by immersion in cold running water until the temperature was below 30 °C. The samples were stored for 6 h at ambient temperature after heat treatment and before any further analysis.

Readjustment of the pH, acidification and rheological (gel strength) measurements

The heated milk samples were carefully re-adjusted to the natural pH (pH 6.7) with 3 M HCl or 3 M NaOH. They were acidified by the hydrolysis of glucono- δ -lactone (GDL Sigma Chemical Co., St Louis, MO, USA) at a 2% (w/w) level (9.8 g milk and 0.2 g GDL) and at 30 °C.

The pH change with time was monitored using a combination glass electrode type InLab 422 (Mettler Toledo, Urdorf, Switzerland) and standard pH meter. The pH gradually changed from pH 6.7 at the start to pH 4.2 after 6 h.

The rheological changes during acidification were monitored with time using low amplitude dynamic oscillation on a standard controlled stress rheometer (e.g a PAAR PHYSICA US 200 rheometer with the Z3 DIN (25 mm) cup and bob arrangement (PHYSICA Messtechnik, GmbH, Stuttgart, Germany) or a Bohlin CVO rheometer and the C25 cup and bob arrangement (Bohlin Instruments UK, Cirencester, Gloucestershire, England)). A typical method has been described in Bikker, J. F., Anema, S. G., Li, Y., & Hill, J. P. *International Dairy Journal*, 10, 723–732, (2000). A description of the storage modulus and other measured values (such as loss modulus, phase angle) are detailed in Ferry (1980) (in: Ferry, J.D. (Ed.), *Viscoelastic Properties of Polymers*, 3rd edn. New York: John Wiley & Sons). After addition of the GDL to the milk and stirring, the appropriate quantity of milk was transferred to the rheometer and the rheological measurements were started. The strain applied was 0.01. The samples were oscillated at a frequency of 0.1 Hz and the temperature of the sample was maintained at 30 °C. Measurements were taken every 5 min for 6 h. The final gel strength was defined as the storage modulus (G') after 6 h of acidification. After 6 h the pH of the samples was 4.2.

Gel strength of the acid gel samples

The samples heated at pH 6.5 had a gel strength (storage modulus) of 166.4 Pa.
The samples heated at pH 6.55 had a gel strength (storage modulus) of 205.1 Pa.
The samples heated at pH 6.6 had a gel strength (storage modulus) of 225.9 Pa.
The samples heated at pH 6.65 had a gel strength (storage modulus) of 241.9 Pa.
The samples heated at pH 6.7 had a gel strength (storage modulus) of 262.2 Pa.
The samples heated at pH 6.9 had a gel strength (storage modulus) of 283.5 Pa.
The samples heated at pH 7.1 had a gel strength (storage modulus) of 309.73 Pa.

These acid gel strength results are summarised in Fig. 1.

Example 2: Skim milk acid gels prepared from reconstituted skim milk with 1.2% added whey protein concentrate (WPC80, equivalent to 1.0 % added whey protein). The gel strength of the acid gels can be varied depending on the pH at heating.

	Weight (g)
Low heat skim milk powder	100g
Whey Protein Concentrate	12g
Water	900g

Milk preparation, pH adjustment and heating

Reconstituted skim milk samples were prepared by adding low heat skim milk powder (100g, whey protein nitrogen index above 6; 37% protein, Fonterra Co-operative Group, New Zealand) to purified water (900g, purified by reverse osmosis followed by filtration through Milli-Q apparatus) to a final concentration of 10% (w/w) total solids. Whey protein concentrate (12g, ALACEN 132, Fonterra Co-operative Group, New Zealand) was added to the milk. The whey fortified reconstituted skim milk samples were allowed to equilibrate at ambient temperature (about 20 °C) for at least 10 h before further treatment.

The pH adjustment, heat treatments, acidification and rheological methodology were the same as described in Example 1.

Gel strength of the acid gel samples from milk samples fortified with 1.2% WPC80

The samples heated at pH 6.5 had a gel strength (storage modulus) between 435 to 451 Pa.
 The samples heated at pH 6.6 had a gel strength (storage modulus) between 417 to 419 Pa.
 The samples heated at pH 6.7 had a gel strength (storage modulus) of 378 Pa.
 The samples heated at pH 6.8 had a gel strength (storage modulus) between 361 to 376 Pa.
 The samples heated at pH 6.9 had a gel strength (storage modulus) between 344 to 346 Pa.
 The samples heated at pH 7.1 had a gel strength (storage modulus) between 330 to 332 Pa.

These acid gel strength results are summarised in Fig. 2, with a comparison with the results from Example 1.

Example 3: Skim milk acid gels prepared from reconstituted skim milk with different levels of added whey protein concentrate. The gel strength of the acid gels can be varied depending on the pH at heating and the level of whey protein added.

	Weight (g)
Low heat skim milk powder	100g
Whey Protein Concentrate	0 to 12g
Water	900g

Milk preparation, pH adjustment and heating

Reconstituted skim milk samples were prepared by adding low heat skim milk powder (100g, whey protein nitrogen index above 6; 37% protein, Fonterra Co-operative Group, New Zealand) to purified water (900g, purified by reverse osmosis followed by filtration through Milli-Q apparatus) at 50°C to a final concentration of 10% (w/w) total solids. Whey protein concentrate (0 to 12g, ALACEN 132, Fonterra Co-operative Group, New Zealand) was added to the milk. The whey fortified reconstituted skim milk samples were allowed to equilibrate at 50°C for at least one hour before further treatment.

After reconstitution, the sample was split into equal portions and pH adjusted using either 1 M NaOH or 1 M HCl to values in the pH range 6.5 to 7.1. After holding overnight at 4°C, the samples were held at 30°C for 30 min and the pH was readjusted. Heat treatment (80°C for 30 min) was conducted in an 80°C shaking waterbath. The sample (150 mL in 500 mL Schott bottles) were placed in the waterbath, and continuously shaken. After 30 min, the bottles were removed from the waterbath and placed in ice/water slurry. The sample was held at 30°C for 4.5 h. The pH of the samples was then adjusted to 6.7 using 1 M NaOH or 1 M HCl. Table 1 summarises the samples that were prepared.

Heated milk (39.2 g) and GDL (0.8g) were added together, stirred for 1 minute, poured into 50 mL plastic containers, and stored at 30°C for 18 h. Each sample was prepared in triplicate.

After the gels were formed, the samples were analysed using a Universal TA-XT2 texture analyser with a real time graphics and data acquisition software package (Stable Microsystems, Haselmare, England) to measure the gel strength. A 10-mm diameter probe was pushed into the acid gel samples (20°C) at a constant rate (1 mm/s) for a set distance (20 mm), and then withdrawn at the same rate. The response was measured as force versus time. The initial force required to penetrate the product, the breaking force and the positive area under the force/time curve were measured. The breaking force was a measure of the acid gel strength .

Table 1 Composition and pH of samples for preparation of acid gels

Sample ID	% RSM	% WPC80	% whey protein	pH
0.0% WPC80 Addition	10	0	0	6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1
0.2% WPC80 Addition	10	0.2	0.16	6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1
0.4% WPC80 Addition	10	0.4	0.32	6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1
0.6% WPC80 Addition	10	0.6	0.48	6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1
0.8% WPC80 Addition	10	0.8	0.64	6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1
1.0% WPC80 Addition	10	1.0	0.8	6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1
1.2% WPC80 Addition	10	1.2	0.96	6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1

Gel strength of the acid gel samples from milk samples fortified with 0 to 1.2% WPC80

There is an interaction between heat treatment pH and WPC80 addition. At low levels of WPC80 addition (<0.4% WPC80 or <0.32% whey protein), higher heat treatment pHs give the highest acid gel strength. However, at WPC80 addition levels above 0.4% (whey protein above 0.32%), this effect reverses, with increasingly lower pH giving higher acid gel strength. Figure 3 summarises the effect of heat treatment pH and WPC80 addition on the final acid gel strength.

As the WPC80 addition level is increased above 0.4% (whey protein above 0.32%), the optimum heat treatment pH steadily decreases. The optimum pH for maximum gel strength was determined and showed that the optimum pH decreased with increasing WPC80 addition. Figure 4 summarises the effect WPC80 addition level on the optimum heat treatment pH.

The above describes some preferred embodiments of the present invention and indicates several possible modifications but it will be appreciated by those skilled in the art that other modifications can be made without departing from the scope of the invention.

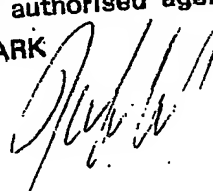
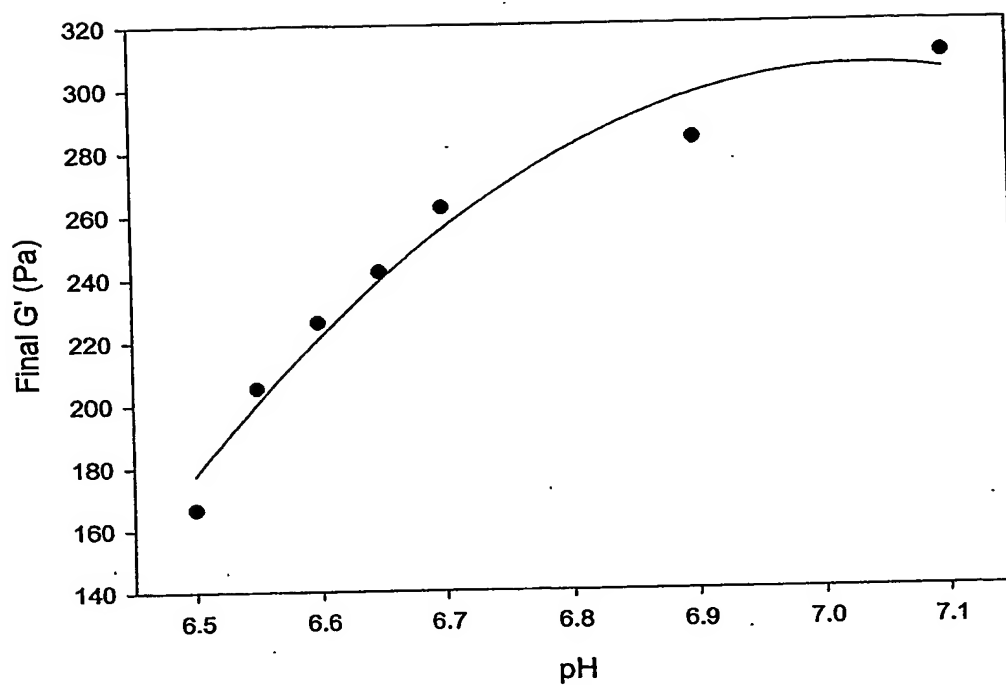
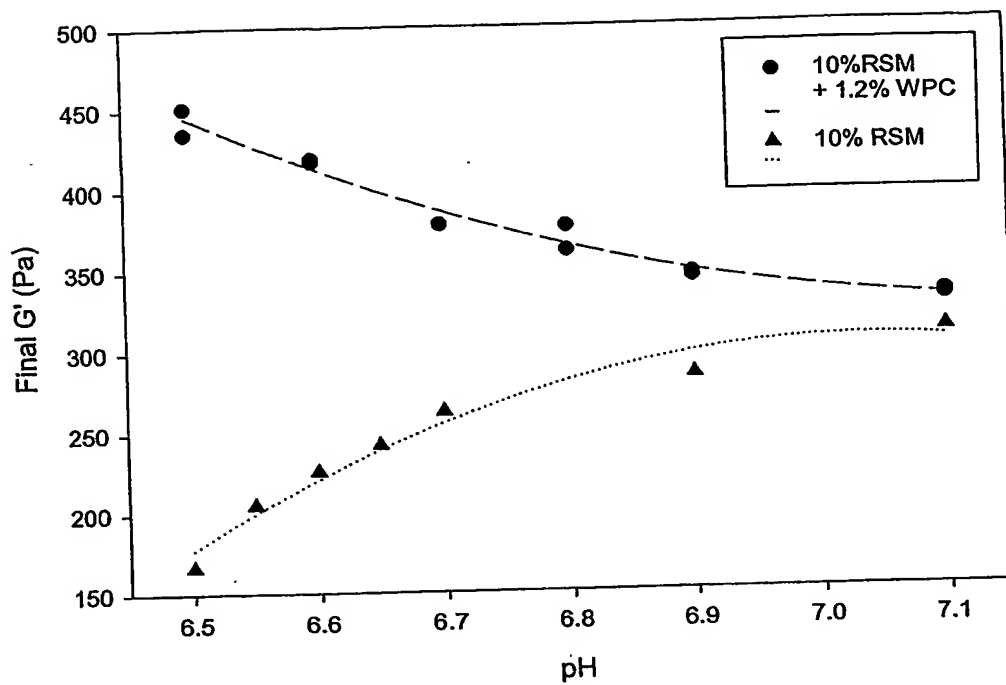
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FIGURE 1



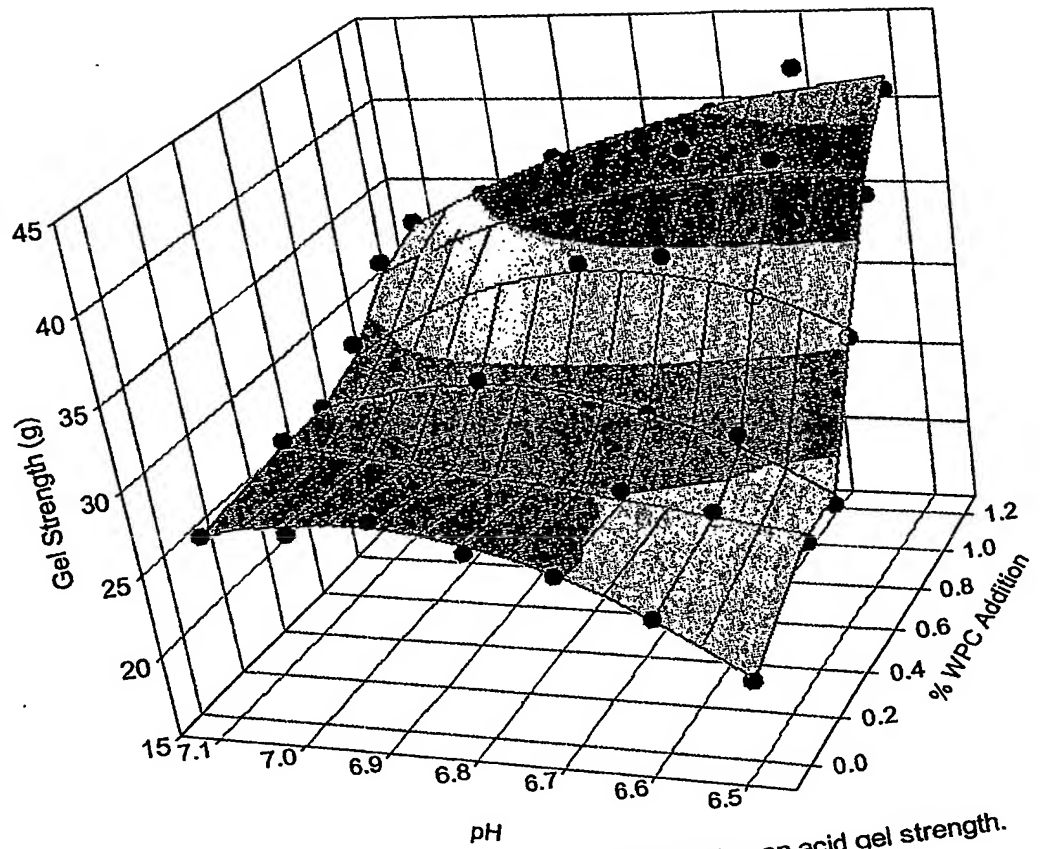
Effect of heat treatment pH on the final acid gel strength for reconstituted skim milk

FIGURE 2

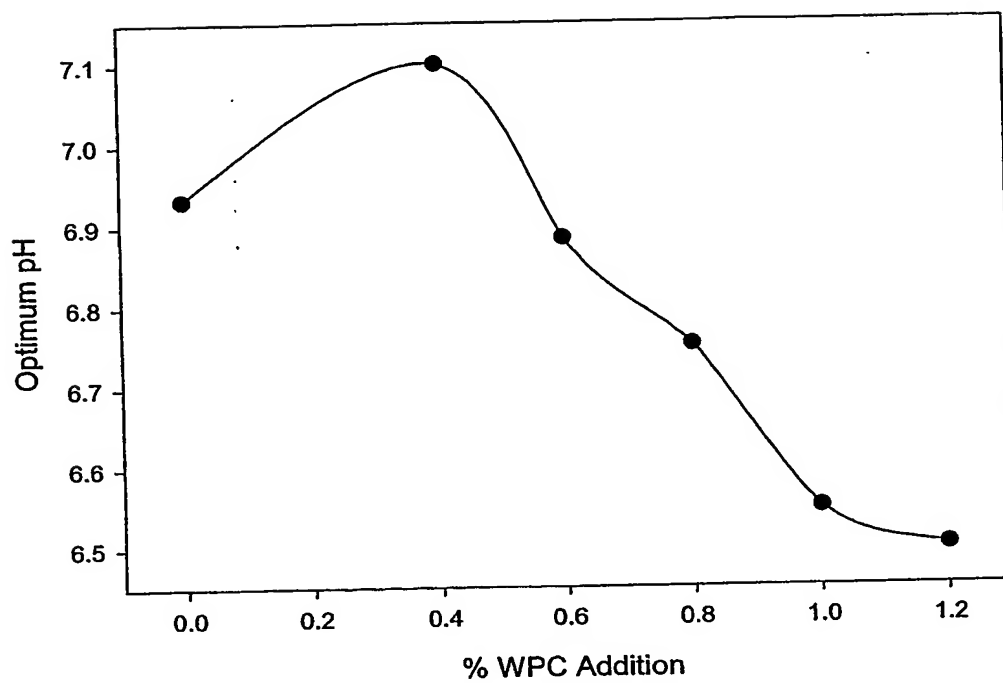


Effect of heat treatment pH on the final acid gel strength for whey-protein-fortified RSM.
(compared to non WPC80 fortified RSM)

FIGURE 3



Effect of heat treatment pH and WPC80 addition on acid gel strength.

FIGURE 4

Effect of WPC80 addition on the optimum heat-treatment pH.

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